5

They want

20

- 1. A method of releasing a protein of interest from host cells comprising contacting the cells with a reducing agent and a detergent, wherein the detergent is an amphipathic charged amine or an amphipathic charged amine oxide.
- 2. The method of claim 1, wherein the detergent is selected from the group consisting of: tributylphosphate, dimethyldecylamine, dimethyltridecylamine, dimethylundecylamine, dimethyldidecylamine, dimethyldecylamine, dimethylhexadecylamine, dimethyldecylamineoxide, dimethylundecylamineoxide, dimethyldidecylamineoxide, dimethyltridecylamineoxide and dimethyltridecylamineoxide.
- 3. The method of claim 1, wherein the detergent is not dimethyltridecylamine.
- 4. The method of claim 1, further comprising the step of adding glycerol to the suspended host cells.
- 5. The method of claim 4, wherein the detergent comprises a final concentration of between 0.01 to 10 percent.
- 6. The method of claim 4, wherein the glycerol comprises a final concentration of between 0.6 to 15

5

- 7. The method of claim 5, wherein the glycerol comprises a final concentration of between 0.6 to 6 percent.
- 8. The method of claim 1, wherein the reducing agent is selected from the group consisting of Dithiothreitol (DTT); Dithioerythiritol (DTE); Cysteine (Cys) and Tris 2-carboxyethyphosphine (TCEP).
- 9. The method of claim 8, wherein the reducing agent is at a concentration of from 0.1 mM to 100 mM.
- 10. The method of claim 1, wherein the host cells are Pichia pastoris cells.
- The method of claim 1, further comprising the step of incubating the cell and detergent and reducing agent mixture from 90 minutes to 24 hours.
- ${\it ll}$ 13. The method of claim 12, wherein the incubation is from 8 hours to 24 hours.

- 23 -

of incubating the cell and detergent and reducing agent mixture at a temperature of between about 3°C and about 10°C.

20